Human Genome Epidemiology (HuGE) Review

Meta-Analysis of the Association of the *Taq1A* Polymorphism with the Risk of Alcohol Dependency: A HuGE Gene-Disease Association Review

Lesley Smith[1], Marion Watson[1], Simon Gates[2], David Ball[3], and David Foxcroft[1]

[1] School of Health and Social Care, Oxford Brookes University, Marston, United Kingdom.
[2] Clinical Trials Unit, Warwick Medical School, University of Warwick, Coventry, United Kingdom.
[3] Social, Genetic and Developmental Psychiatry Centre (MRC), Institute of Psychiatry, King's College London, London, United Kingdom.

The human dopamine 2 receptor *Taq1A* allele has been implicated as a vulnerability factor for alcohol dependence in a number of studies and reviews. To determine whether this allele is associated with alcoholism, the authors conducted a Human Genome Epidemiology review and meta-analysis. Forty-four studies with 9,382 participants were included. An odds ratio of 1.38 (95% confidence interval: 1.20, 1.58; heterogeneity, 50.5%) was found for the *A1A1 + A1A2* versus the *A2A2* genotype. Sensitivity analyses suggested lack of ethnic matching as a possible source of heterogeneity; a small, significant association was detected in studies with ethnic-matched controls (odds ratio = 1.26, 95% confidence interval: 1.02, 1.56; heterogeneity, 37%). Significant associations were also found in analyses restricted to studies reporting use of blinding and those with adequate screening of controls for alcohol dependency. For the *A1A1* versus the *A1A2 + A2A2* genotype, the odds ratio was 1.22 (95% confidence interval: 1.05, 1.43; heterogeneity, 0%). Sensitivity analyses on groups of studies reporting use of ethnic-matched controls and those that screened controls for alcohol dependency still showed significant associations. The relatively small effect for the association of the *A1* allele, or another genetic variant linked to it, with alcohol dependence indicates a multigene causality for this complex disorder.

alleles; association; dependency (psychology); *DRD2*; epidemiology; meta-analysis; review (gene-disease association); *Taq1A*

Abbreviations: CI, confidence interval; *DRD2*, dopamine receptor D2 gene; GABA, γ-aminobutyric acid; OR, odds ratio.

Editor's note: This paper is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/).

GENE

Alcohol is known to increase dopaminergic function in the mesolimbic system, a brain reward system thought to be crucial in drug-mediated reinforcement behavior, and therefore may be involved in the pathogenesis of alcohol dependence. The dopamine receptor D2 gene (*DRD2*) has been one of the most extensively studied in addictive disorders, with the *Taq1A* polymorphism being the most frequently studied. The *DRD2 Taq1A* polymorphism is located more than 10 kilobase-pairs downstream from the coding region of the *DRD2* gene at chromosome 11q23, and a mutation in this noncoding region would not be expected to produce a structural change in the dopamine receptor (1, 2). Therefore, the functional significance of the polymorphism is

Correspondence to Dr. Lesley Smith, School of Health and Social Care, Oxford Brookes University, Jack Straws Lane, Marston, OX3 0FL, United Kingdom (e-mail: lesleysmith@brookes.ac.uk).

unclear. It is suggested that the *Taq1A* polymorphism may be in linkage disequilibrium with an upstream regulatory element or another functional gene that confers susceptibility to alcoholism. More recently, this *DRD2*-associated polymorphism has been more precisely located within the coding region of a neighboring gene, *ANKK1* (ankyrin repeat and kinase domain containing 1), which may confer a change in the amino acid sequence (3).

DISEASE

DRD2 has been implicated as a vulnerability factor for alcohol dependence in a number of studies and previous reviews. Blum et al. (4) were the first to report a significantly higher frequency of the *A1* allele of the *Taq1A* polymorphism near the *DRD2* gene in alcoholics compared with nonalcoholic controls. This finding suggests an increased susceptibility to alcohol dependence in people with a particular variant of the *DRD2* gene. Later studies supported this initial finding (5–7). Other studies, reviews, and meta-analyses, however, have generally been less positive about the evidence for an association between *DRD2* and alcohol dependence (1, 8–10) because more robust association methods did not support the original report. It has been suggested that the nature of the control group may determine whether significant population-based associations are found (6). Therefore, the association of the *DRD2 Taq1A* allele with alcohol dependence remains unclear and controversial.

The *Taq1A* allele has also been implicated in other addictive disorders such as polysubstance abuse (11); cocaine (12–14), opioid (15), methamphetamine (16, 17), and nicotine dependence (18); and gambling (19) as well as in other mental health disorders such as mood disorders, schizophrenia, and posttraumatic stress disorder (20, 21). However, no conclusive linkage or association has been found. Other candidate genes (alleles) implicated in alcohol dependency are the alcohol-metabolizing enzymes alcohol dehydrogenases and acetaldehyde dehydrogenase, and γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the human brain (22). GABA acts via two receptor types, A and B. $GABA_A$ receptors are activated by benzodiazepines, which have pharmacologic properties similar to alcohol; thus, $GABA_A$ receptor genes are strong candidates. Several association studies point to the involvement of $GABA_A$ receptor subunit genes clustered on chromosome 5 and the development of alcohol dependence (23–29).

Prevalence data from population surveys in the United States have shown that about 6 percent of men and 2 percent of women are classified as alcohol dependent (30). Although a predisposition to alcohol dependency is thought to be partly attributable to genetic factors, a role for other risk factors has also been suggested. Previous work has linked alcohol abuse and dependence to an earlier age at drinking onset (31–38); being male (31, 33); being divorced, separated, or never married (31); having an early history of antisocial behavior (33, 39, 40); and belonging to a lower socioeconomic group (41). However, these findings are mostly from cross-sectional surveys, which are susceptible to recall bias, and there are few data confirming the association between specific risk factors and alcohol dependency from robust prospective studies with sufficient duration of follow-up and adequate control for confounding (31).

OBJECTIVES

The aim of this Human Genome Epidemiology review was to systematically review and perform a meta-analysis of all available evidence from observational studies regarding the association of the *DRD2 Taq1A* allele with alcohol dependence.

METHODS

Inclusion criteria

Studies were selected if they evaluated an association between the *Taq1A* allele of the *DRD2* gene and alcohol dependency and included a non-alcohol-dependent concurrent control group. Only studies published in the English language were considered.

Identification and eligibility screening of studies

We identified eligible studies by searching Medline (National Library of Medicine, Bethesda, Maryland), Embase (Elsevier, Amsterdam, the Netherlands), and BIOSIS (Thomson Scientific, Stamford, Connecticut) from their inception to August 2006. We used the following thesaurus search terms (exploded): ''alcohol-related disorders'' OR ''alcoholic beverages'' OR ''alcoholic drinking'' combined with the free-text terms ''allel*'' OR ''gene'' OR ''genes'' OR ''locus'' OR ''loci'' OR ''receptor*'' OR ''genotyp*''OR ''polymorphi*'' OR ''RFLP'' OR ''genetic*'' OR ''mutation*'' OR ''variant*'' AND ''alcohol*'' OR ''drink*'' AND ''DRD2'' OR ''*Taq*1A.'' Two reviewers (L. S. and M. W.) screened the title and abstract of each electronic citation; full-text copies of potentially relevant studies were obtained. Each full-text copy was screened for eligibility (L. S. and M. W.). References from retrieved study reports and reviews were also screened for additional studies.

Data extraction

Two investigators (L. S. and M. W.) independently extracted data by using a structured form. Discrepancies were resolved by discussion and consultation with a third reviewer (D. F.). The following information was sought from each report: selection and diagnostic criteria of the alcohol-dependent group; selection and classification criteria of the control group; demographic information including ethnicity, age, and sex; all alleles investigated; method of ascertainment of genotype; blinding of personnel performing genotyping to clinical status of the study participants; methods used to create balanced groups (matching procedures or statistical adjustment methods); genotype and allelic frequencies; and statement of Hardy-Weinberg equilibrium. Studies were categorized as Caucasian based on the original study's use of the term to describe ethnic group or for studies conducted on White North Americans or Europeans.

Data analysis

We estimated unadjusted odds ratios for published genotype frequencies. Pooled odds ratios were calculated by using a random-effects model (42) stratified by geographic region/ethnicity. Geographic regions/ethnic groups were Caucasian, Native American, Japanese, Chinese, Hispanic, Indian, and Korean. Studies with mixed populations were added to the group that represented the majority of the participants. We quantified the extent of heterogeneity by using I^2, which represents the proportion of variability between studies attributable to true variability rather than chance (43). There are several approaches to analyzing gene-disease association studies. We conducted two analyses; one assumed a dominant model of gene action (homozygous wild type vs. heterozygous and homozygous variant), the other a recessive model of gene action (homozygous wild type plus heterozygous vs. homozygous variant). We chose not to compare allele frequencies (codominant model) between cases and controls, because this process double-counts people, or to perform several pair-wise comparisons, because each pair-wise comparison leaves out valuable information. In addition, both analyses increase the likelihood of a type 1 error. We tested whether the genotype frequencies in the controls were in agreement with the expected distribution (Hardy-Weinberg equilibrium) by using Pearson's χ^2 with 1 degree of freedom, with Yates's correction for cells with values of less than 5.

We investigated heterogeneity through a number of preplanned sensitivity analyses in which studies were eliminated from analyses if they significantly deviated from Hardy-Weinberg equilibrium, did not use ethnic-matched controls, did not report use of blinding of case-control status and/or genotyping, and did not report adequate screening of control groups to exclude alcohol dependency. Cumulative meta-analyses were also performed. We used Stata software, version 8 (Stata Corporation, College Station, Texas) for cumulative meta-analyses and Hardy-Weinberg analysis, and the computer program RevMan, version 4.1 (The Nordic Cochrane Centre, The Cochran Collaboration, Copenhagen, Denmark) for all other analyses.

RESULTS

Description and quality of included studies

We screened 1,056 titles and abstracts and obtained 116 full-text papers. Of these, 44 met our eligibility criteria. We included 44 studies with a total of 9,382 participants. Forty-two were case-control studies, and two were cross-sectional surveys (44, 45). Characteristics of included studies are summarized in table 1. Three studies reported allelic frequencies only (6, 46, 47), but we were able to extract genotype frequencies from a review published by Noble (48) for the Bolos et al. (46) and Neiswanger (6) studies. All others reported genotype frequencies.

Alcohol dependency was determined by using different diagnostic criteria: the *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition, Revised; *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition,

Revised; *International Statistical Classification of Diseases and Related Health Problems*, Tenth Revision; and Feighner criteria (precursor to modern psychiatric classification criteria). Two studies also used autopsy-confirmed alcohol misuse cases (4, 49). Fourteen studies excluded patients with other addictions and major psychiatric disorders (5, 46, 50–61). Controls were classified as not alcohol dependent, but the thoroughness of this diagnosis varied across studies, and some studies may not have screened the controls adequately to eliminate possible cases (45–47, 56, 60, 62–71). Blinding of clinical investigators assessing case-control status to genotype was reported by only 10 studies (4, 5, 45, 46, 56, 57, 72–75).

Matching for sex was described in 10 studies (44, 51, 52, 59, 64, 69, 74, 76–78), for age in two (76, 77), and for ethnicity in 30 (5, 6, 44–46, 49, 52, 54, 56–60, 62, 64, 66–70, 75–84). Few (nine) studies reported blinding to case or control status of personnel who performed the genotyping (4, 5, 52, 56, 57, 62, 65, 75, 85). Polymerase chain reaction was used for genotyping in 25 studies (5, 45, 49, 50, 54, 57–61, 63, 64, 67, 69–71, 73, 74, 76–79, 81, 86, 87) and hybridization in 16 studies (4, 6, 46, 51–53, 55, 56, 62, 65, 66, 68, 72, 75, 84, 85); three did not report the method used (44, 47, 83). Genotyping was conducted in duplicate by only six studies (52, 57, 62, 67, 75, 76); in one study (87), 10 percent of the sample was checked; and, in one study, samples were genotyped in triplicate (4).

Overall effects

Forty-three studies, including a total of 5,273 cases and 3,995 controls, reported genotype frequencies for the *Taq1A* polymorphism and alcohol dependency.

We found evidence suggesting that genotypes in control groups were not in Hardy-Weinberg equilibrium ($p < 0.05$) in three studies (46, 68, 74).

For all studies combined, when we assumed the dominant model of gene action (*A1A1* + *A1A2* vs. *A2A2*), a small but significant association of alcohol dependency with being homozygote or heterozygote for the *A1* allele was detected. The odds ratio was 1.38 (95 percent confidence interval (CI): 1.20, 1.58) when random effects were used, although substantial statistical heterogeneity was detected between studies ($I^2 = 50.5$ percent, $p = 0.0001$). Stratifying the studies into subgroups by ethnic group produced similar results, with significant associations detected in the two largest subgroups: Caucasian (odds ratio (OR) = 1.57, 95 percent CI: 1.29, 1.91) with substantial heterogeneity ($I^2 = 57.2$ percent, $p = 0.0001$) and Chinese (OR = 1.35, 95 percent CI: 1.04, 1.75) with no heterogeneity ($I^2 = 0$ percent, $p = 0.71$) (Web figure 1; this information is shown in the first of four supplementary figures; each is referred to as "Web figure" in the text and is posted on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/reviews.htm) as well as on the *Journal*'s website (http://aje.oupjournals.org/)).

Pooling the results of the same studies, but assuming the recessive model of gene action (*A1A1* vs. *A1A2* + *A2A2*), also showed a small but significant positive association of

TABLE 1. Characteristics of studies included in a meta-analysis of the association of the *Taq1A* polymorphism with the risk of alcohol dependency

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (*p* value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Amadeo, 2000 (86)	Polynesian, Polynesian/Caucasian, Polynesian/Chinese hospitalized for alcohol dependence; 38 with cannabis abuse/dependence; DSM-III-R* criteria; interview; $n = 71$ (61 men, 10 women); mean age, 40.8 years	Volunteers; similar ethnicity; none abused alcohol, cannabis, nicotine, or other drugs; $n = 59$ (26 men, 33 women); mean age, 29.5 years	*Taq1A*, PCR*; no information on blinding	0.104
Anghelescu, 2001 (50)	German alcohol dependents; DSM-IV* criteria; admitted for detoxification; 56% nicotine dependent; M-CIDI,* interview, and ASI* conducted by psychiatrist; psychotic disorders and substance abuse other than nicotine excluded; $n = 243$ (187 men, 56 women)	Unrelated healthy volunteers; no depressive, drug abuse, or alcohol dependence; M-CIDI criteria; 1% nicotine dependent; $n = 98$ (72 men, 26 women)	*Taq1A*, PCR; no information on blinding	0.436
Arinami, 1993 (51)	Japanese alcoholics; hospital inpatients; randomly selected; unrelated; DSM-III-R criteria; assessed by psychiatrist; major affected disorder, schizophrenia, or psychoses excluded; interview for family history of alcoholism, $n = 78$ (74 men, 4 women); mean age, 51 (range, 25–79) years	Group 1: unrelated volunteers selected from staff, students, and routine patients and relatives of patients; unscreened, $n = 100$; mean age, 44 (SEM,* 1.2) years; group 2: gender-matched, nonalcoholics recruited from care staff; did not meet criteria for alcoholism; nonsmokers; $n = 35$; mean age, 45 (SEM, 1.5) years	*Taq1A*; hybridization; no information on blinding	0.096
Bau, 2000 (63)	Unrelated Caucasian, alcoholic, Brazilian hospital detoxification patients; DSM-III-R criteria and interview; $n = 115$; males; mean age, 41 (range, 20–63) years	Selected from paternity testing clinic; unscreened for alcoholism; $n = 114$ (57 men, 57 women)	*Taq1A*, PCR; no information on blinding	0.572
Blum, 1990 (4)	Caucasian cadavers of alcoholics; cause of death related to alcohol abuse; samples obtained from National Neurological Research Bank, United States; alcohol dependence or alcohol abuse or nonalcoholic status determined independently by two psychiatrists using medical records and relative or treatment center staff, 100% agreement; $n = 35$ (32 men, 3 women); 22 White, 13 Black; mean age, 51.0 (SEM, 2.3) years	Cadavers of nonalcoholics; samples obtained from National Neurological Research Bank, United States; $n = 35$ (28 men, 7 women); 24 White, 11 Black; mean age, 51.9 (SEM, 2.5) years	*Taq1A*; hybridization; three independent samples blind to clinical status	0.181
Blum, 1991 (72)	Caucasian alcoholics receiving treatment for alcohol dependence; volunteers; living in Texas; classified as severe (dependent with medical complications) or less severe (dependent only) based on three questionnaires, DSM-III-R criteria for alcohol dependence and abuse; $n = 96$ (65 men, 31 women); 89 White, 7 Black; mean age, 44.8 (SEM, 1.4) years	Nonalcoholic volunteers from treatment center or hospital staff; $n = 43$ (19 men, 24 women); 31 White, 12 Black; mean age: 42.0 (SEM, 2.4) years; all diagnoses for cases and controls made by two independent interviewers with no knowledge of genotype	*Taq1A*; hybridization; no information on blinding	0.463
Bolos, 1990 (46)	Unrelated Caucasian alcoholics all admitted to NIAAA* unit; DSM-III-R and RDC* criteria for alcohol dependence by two independent clinical interviewers blind to genotype status; no major psychiatric disorder or major drug abuse; $n = 40$ (36 men, 4 women); mean age, 44 (SEM, 2) years	Unrelated White patients with cystic fibrosis ($n = 62$) and unrelated White patients from family pedigree studies ($n = 65$)	*Taq1A*; hybridization; no information on blinding	0.024

Table continues

TABLE 1. Continued

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (*p* value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Chen, 1996 (81)	Atayal Taiwanese (aboriginal); alcohol dependent; DSM-III-R criteria and Chinese diagnostic interview, alcoholism section; *n* = 85; mean age, 40 years	Nonalcoholics; Atayals from the same community; clinical status determined by interview; *n* = 41 (15 men, 26 women)	*Taq1A*, PCR; no information on blinding	0.510
Chen, 1997 (64)	Han Chinese and four aboriginal groups in Taiwan from community and clinical settings, DSM-III-R criteria for alcohol dependence; assessed by psychiatrist or trained Ras, semistructured clinical interview, defined as severe by liver function, peripheral neuropathy and hallucinations *n* = 203, 178 men 35 women; age 41.2–51.1 years	Never or occasional drinkers, matched for gender and ethnicity, unrelated; from community and hospital, *n* = 213, 178 men, 35 women; age 54.2–61 years	*Taq1A* and *NcoI*; PCR + *Taq* digestion	0.772
Comings, 1991 (65)	North American; Caucasian; attending an addiction clinic; DSM-III-R criteria for alcohol dependence and an additional 13 cases from brain tissue from NNRB*; *n* = 104	Nonpsychiatric genetic and chromosome abnormalities genetic cell repository, New Jersey, *n* = 19; brain samples from NNRB, *n* = 12; laboratory personnel and patients with nonpsychiatric genetic disorders, *n* = 8; unrelated individuals in CEPH* families, *n* = 69; only groups 2 and 3 known to be nonalcoholics, *n* = 108	*Taq1A*; hybridization; genotype read blind to clinical status	0.177
Cook, 1992 (52)	Male Caucasian alcoholics in a VA* alcohol treatment unit in nonurban areas of Iowa and neighboring states according to DSM-III-R criteria obtained by structured interview; no other substance dependence or psychiatric conditions; *n* = 20; mean age, 56.9 (SD,* 15.3) years	Nonalcoholics selected from hospital employee volunteers; sex, race, and geographic region matched; interviewed by questionnaire (Brief MAST*) to assess alcohol-related problems; anonymous to encourage honesty; *n* = 20; all men; mean age, 32.6 (SD, 4.6) years	*Taq1A*; duplicate genotyping by hybridization; blind to clinical status	0.451
Cruz, 1995 (53)	Unrelated male Mexican alcoholics hospitalized for hallucinosis, withdrawal symptoms, or delirium tremens; DSM-III-R and AUDIT* and Eysenck Personality Questionnaire, major mental disorders excluded except major depression or psychoactive substance abuse; *n* = 38; mean age, 34 (range, 21–47) years	Hospital staff matched on ethnicity; positive family history excluded; *n* = 38 (27 men, 11 women); mean age, 30.8 (range, 20–50) years	*Taq1A*; hybridization; no information on blinding	0.192
Finckh, 1996 (54)	Unrelated Caucasian alcoholics of German origin recruited from three different sources: hospital detoxification unit, homeless not receiving treatment, hospitalized in a psychiatric addiction unit; ICD-10* criteria for alcoholism; structured interview; other drug addictions and major psychiatric comorbidities excluded; *n* = 312 (273 men, 39 women); mean age, 42 (SD, 9.4) years	Ethnically matched, nonrelated individuals without neuropsychiatric disorders or drug and alcohol dependence; *N* = 131 (61 men, 70 women); mean age, 54 (SD, 16) years	*Taq1A*, and exon 7 *C/G Ser/Cys* polymorphism PCR; no information on blinding	0.698
Foley, 2004 (49)	Brain tissue collected at autopsy from Caucasian alcoholics, 34% with cirrhosis; alcohol-related end-organ damage confirmed pathologically at autopsy; alcohol consumption, ascertained by medical records, in excess of 80 g/day most of adult life; *N* = 87	Unrelated Caucasians, none with a history of alcoholism or liver disease; no clinical or pathologic evidence of neurologic disease; *N* = 109	*Taq1A*, *Taq1B*, *ADH1C*, *EA AT2G 603A*, *5HTTL-PR*, *NMDAR2B*, *DAT1 SLC6A3*, PCR; no information on blinding	0.570

Table continues

TABLE 1. Continued

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (p value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Geijer, 1994 (55)	Scandinavian alcoholics from an inpatient detoxification program; DSM-III-R criteria plus interview; excluded if history of psychiatric disorders or dependence on psychoactive substances other than alcohol; criminal and social security registers used to verify alcoholism and exclude mental/nervous disorders; psychoactive substance detection at autopsy excluded; N = 74 (64 men, 10 women); mean age, 46 (range, 29–71) years; plus 19 autopsy samples from alcoholics (18 men, 1 woman); mean age, 51.1 (range, 25–75) years	Persons screened for alcohol dependence or abuse; DSM-III-R criteria and medical record review to verify clinical status and exclude those with substance misuse; N = 81 (43 men, 38 women) mean age, 39.5 (range, 29–56) years	Taq1A, Taq1B; hybridization; no information on blinding	0.470
Gelernter, 1991 (56)	Unrelated Caucasian alcohol-dependent subjects participating in pharmacotherapy trials for alcoholism; no comorbid psychiatric disorder or opiate abuse or dependence; DSM-III-R criteria and structured clinical interview; diagnosis blind to genotype status; N = 44 (34 men, 10 women); mean age, 40.5 (range 23–62) years	Unrelated White patients from family pedigree studies, mixed European ancestry, described as a random sample; no psychiatric interview; N = 68	Taq1A; hybridization; genotyping blind to case status	0.662
Gelernter, 1999 (83)	European Americans seeking treatment for substance misuse; DSM-III-R criteria: 7–9 criteria met = severely affected, 3–6 = affected; n = 160	European Americans screened by various questionnaires including DSM-III-R and SADS-L* or interview to exclude substance use and psychotic, anxiety; and mood disorders; N = 136	Taq1A, Taq1B, Taq1D, and −141C InsDel; genotyping methods described in Gelernter (98); no mention of blinding	0.423
Goldman, 1992 (66)	Finnish violent alcoholics imprisoned, unrelated, and with Finnish parents; N = 46; all male; mean age, 29.2 (SEM, 1.3) years	Finnish nonalcoholics, unrelated with Finnish parents; paid volunteers recruited through newspaper advertisements; N = 36; all male; mean age, 30.1 (SEM, 2.5) years	Taq1A; hybridization; no information on blinding	0.570
Goldman, 1997 (45)	Southwestern American Indian population, interrelated pedigrees; DSM-III-R criteria for alcohol dependence, substance misuse (excluding alcohol), or schizophrenia; clinical interview blind to genotype; N = 459 (121 women, 155 men) with alcohol dependence	N = 161 unaffected from the same sample population as cases	ser311cys, Taq1A, intron 2 STR,* PCR; no information on blinding	0.284
Gorwood, 2000 (57)	Males alcoholics of French origin from the psychiatric department and outpatient clinic of two general hospitals; DSM-III-R criteria plus interview; schizophrenia and dementia excluded; analysis blinded; n = 113; mean age, 43.6 (SD, 10.3) years	Male blood donors of French origin; no alcohol dependence; DSM-III-R criteria, classified as social drinkers; n = 49; minimum age, 35 years	Taq1A, PCR; duplicate genotyping blind to clinical status	0.207
Heinz, 1996 (58)	Unrelated individuals, German nationality of European descent; alcohol dependent in a detoxification outpatient clinic; ICD-10 criteria; no comorbid psychiatric disorders; no other substance misuse; N = 97	Nonalcoholics; ethnically matched; at least age 26 years; no addictive disorder or previous psychiatric treatment ascertained by questionnaire; N = 113 (50 men, 63 women)	Taq1A, PCR; no information on blinding	0.787
Hietala, 1997 (84)	Finnish unrelated male alcoholics in a detoxification program; DSM-III-R criteria for alcohol dependence; clinical interviews; n = 70, mean age, 42.1 (SD, 8.5) years	Unrelated male volunteers from two industrial corporations in Finland; screened to exclude alcohol abuse and major mental disorders by interview and medical record review; n = 50; mean age, 44.1 (SD, 8.3) years	Taq1A; hybridization; no information on blinding	0.209

Table continues

TABLE 1.   Continued

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (p value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Ishiguro, 1998 (59)	Unrelated, Japanese alcoholic patients diagnosed according to DSM-III-R criteria for alcohol dependence and withdrawal symptoms; other addictions excluded; $N = 209$ (195 men, 14 women); mean age, 52.0 (range, 29–75) years	Unrelated, healthy age- and sex-matched Japanese with no history of addictive disorder or psychiatric treatment determined by questionnaire; $N = 152$ (137 men, 15 women); mean age, 50.8 (range, 25–65) years	−141C Ins/Del and *Taq1A*, PCR; no information on blinding	0.317
Karaoguz, 2004 (74)	Turkish, unrelated alcoholic males, severe drinkers of Raki (45% ethanol) per DSM-IV criteria; psychiatrists blind to genotype; type II characterized by fighting when drinking, automobile accidents, arrest for reckless driving, hospitalization for alcoholism, and inability to abstain before aged 25 years; $N = 52$; males; mean age, 43 (range, 28–63) years	Healthy males; no psychiatric or organic diseases; no history of alcohol or drug dependency; $N = 93$; males; mean age, 29.8 (range, 17–50) years	*Taq1A*, PCR; no information on blinding	<0.001
Konishi, 2004 (87)	Mexican Americans living in Los Angeles, California, recruited from human service agencies, treatment programs, mental health clinics, AA* groups, Hispanic churches and organizations, and medical emergency rooms; DSM-IV criteria for alcoholism; structured clinical interview; $N = 200$; mean age, 39.0 (SD, 10.8) years	Nonalcoholics; structured clinical interview; $N = 251$; mean age, 32.5 (SD, 9.2) years	*Taq1A*, *Taq1B*, intron 6, intron 7, −141C ins/del, *ADH1B*, *ADH1C*, *ALDH2*, PCR for *ADH* genotypes, 10% of each genotype duplicated; no information on blinding	0.797
Kono, 1997 (73)	Native Japanese; unrelated; DSM-III-R criteria: 7–9 criteria met = severely affected, 3–6 = affected; subtyped by age at onset (<25 years) and family history of alcohol dependence; also by mood disorder, hallucinosis, withdrawal delirium, or seizures; two independent psychiatrists blind to genotype; $n = 100$ (78 men, 22 women); mean age, 48.7 (SD, 9.4) years	Unrelated; alcoholism and family history of alcoholism excluded; $n = 93$ (70 men, 23 women); mean age, 42.5 (SD, 13.3) years	*Taq1A*, PCR; no information on blinding	0.183
Lawford, 1997 (5)	Unrelated Australian volunteers of Caucasian descent, age not given; sample A: $N = 68$ (63 men, 5 women admitted to an acute detoxification unit); sample B: $N = 90$ (85 men, 5 women admitted to a psychiatric unit for alcohol rehabilitation); sample C: $N = 43$ (36 men, 7 women admitted for alcohol-induced gastrointestinal disease but excluded if they had dementia, delirium, psychosis, or other CNS* impairment); fulfilled DSM-III-R criteria for alcohol abuse/ dependence; detailed interview and questionnaire; all interviews blind to genotype status	Unrelated Australian volunteers of Caucasian descent; hospital staff; nonsmokers; no current daily alcohol consumption above 6 g for men and 40 g for women; structured interview; $N = 46$ (16 men, 30 women)	*Taq1A*, PCR; genotyping blind to clinical status	0.315
Lee, 1999 (71)	Chinese Han with alcohol dependence: 19 hospitalized with withdrawal symptoms or medical complications of alcoholism, 109 outpatients with complications of alcoholism; DSM-III-R–defined alcohol dependence; structured clinical interview; $N = 128$ (120 men, 8 women); mean age, 38.11 (SD, 10.78) years	Nonalcoholics; unrelated; selected from community and hospital; same ethnic group; $N = 85$ (75 men, 10 women); mean age, 36.95 (SD, 12.32) years; 19 alcoholics and 24 nonalcoholics included in earlier Lu study (68)	*Taq1A*, *Taq1B*, *ADH2*, *ADH3*, and *ALDH2*, PCR, duplicate tests; no information on blinding	0.361

Table continues

TABLE 1. Continued

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (*p* value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Limosin, 2002 (79)	French Caucasian, alcohol-dependent, detoxification inpatients; DSM-III-R criteria plus interview; *n* = 120: 62 men (mean age, 44.4 years), 58 women (mean age, 46.3 years)	Ethnically matched; DSM-III-R interview; no history of addictive disorder, alcohol/substance, or abuse/dependence; *n* = 107 (66 men, 41 women); aged >18 years	*Taq1A*, PCR; no information on blinding	0.786
Lu, 1996 (68)	Taiwanese male alcoholics from three ethnic groups; Chinese Han: *n* = 21, Atayal (aboriginal): *n* = 21, Ami (aboriginal): *n* = 20; some related individuals in both groups because of small communities; DSM-III-R criteria for "severe" alcohol dependence based on clinical interview	Taiwanese male nonalcoholics matched on ethnic group and geographic area; Chinese Han: *n* = 25, Atayal: *n* = 21, Ami: *n* = 20	*Taq1A*, *Taq1B* and intron 2 STR polymorphism; hybridization, no information on blinding	0.011
Lu, 2001 (60)	Han Chinese in Taiwan hospital patients; DSM-III-R–defined alcohol dependence; clinical interview plus interview with family member to confirm diagnosis; major mental illness and conduct disorder excluded; *n* = 97 (88 men, 9 women)	Han Chinese in Taiwan; community and medical staff; unrelated; *n* = 85 (75 men, 10 women)	*Taq1A* and *Taq1B*, PCR; no information on blinding	0.361
Lee, 1997 (67)	Korean alcoholics selected from two hospitals; DSM-III-R criteria; *N* = 67; male; mean age, 46 (SD, 9.28) years; range, 27–70 years	No history of alcoholism; *N* = 100; male; mean age, 45 (SD, 8), range, 27–67 years	*Taq1A*, PCR; no information on blinding	0.235
Matsushita, 2001 (77)	Japanese inpatients hospitalized for 2–4 weeks for alcohol dependence or abuse, DSM-III-R criteria, clinical interview, 376 with active *ALDH2*, 74 with inactive *ALDH2*; plus 133 male alcoholics from detoxification unit, DSM-III-R, with inactive *ALDH2*; *n* = 583 (541 men, 42 women); mean age, 50.2 years	Unrelated Japanese volunteers; age and gender matched; mainly hospital employees or acquaintances; drinking behavior evaluated by KAST*; potential drinkers excluded; *n* = 278 (252 men, 26 women); mean age, 51.4 years	*Taq1A*, PCR; no information on blinding	0.249
Neiswanger, 1995 (6)	Unrelated Caucasian alcoholics; DSM-III-R plus Feighner diagnostic interview schedule; current and lifetime history of drinking and drug use; 20% also drug dependent; psychopathologies minimal; *n* = 52 (20 men, 32 women); mean age, 33.8 (SD, 5.7) years	Unrelated Caucasian controls from "low-risk" families; screened for alcoholism and other psychopathology; *N* = 30; mean age, 58.3 (SD, 12.1) years	*Taq1A*, hybridization; no information on blinding	0.449
Noble, 1994 (85)	Caucasian, hospitalized alcoholics with alcohol-induced chronic organ disease, SAAST* score at least 11, and DSM-III-R criteria for alcohol abuse/dependence; Severity of Alcohol Dependence Questionnaire also used; drug abuse/dependence not excluded; *N* = 73 (63 men, 10 women); 44 Caucasian, 21 Hispanic, 8 Black; mean age, 44.0 (SD, 1.0) years	Hospitalized patients with chronic organ disease, no history of alcoholism per DSM-III-R, and SAAST score no more than 5; *N* = 80 (60 men, 20 women); 58 Caucasian, 11 Hispanic, 11 Black; mean age, 55.8 (SD, 1.2) years	*Taq1A*, hybridization; genotyping blind to clinical status	0.630
Ovchinnikov, 1999 (69)	Slavic-surnamed patients with alcohol dependence; group 1: age at onset >25 years, negative family history, less severe disease; group 2: age at onset <25 years, family history positive, severe alcohol abuse; according to ICD-10 criteria, group 1: *n* = 18, group 2: *n* = 24; men; mean age; 39.8 (SD, 10.3) years	Gender matched, nonalcoholics recruited from staff members of authors' institutions; Slavic origin; *N* = 76; mean age, 31.6 (SD, 5.3) years	*Taq1A*, *Taq1B*, exon 3 DRD4, PCR; no information on blinding	0.561

Table continues

TABLE 1. Continued

Study (first author, year (reference no.))	Participant characteristics		Genotype and method	Hardy-Weinberg (*p* value)
	Alcohol-dependent cases	Non-alcohol-dependent controls		
Parsian, 1991 (75)	Unrelated alcoholics taking part in a larger alcoholism study; Caucasian; diagnosed according to Feigner criteria; diagnosis blind to genotype status; $N = 32$; 81% men; mean age, 34.6 (SD, 7.6) years	Unrelated Whites who did not meet Feigner or Spitzer criteria for alcoholism; $N = 25$; 36% men; mean age, 51.8 (SD, 11.7) years	*Taq1A*, hybridization; genotyping in duplicate, blind to case status	0.441
Pastorelli, 2001 (76)	White Italians; alcohol dependent; seeking treatment or admitted for medical reasons; 80% smokers; 27% polysubstance abusers; interview; $N = 60$ (45 men, 15 women); mean age, 46.4 (SD, 10.8) years	Blood donors matched on sex and age; non-alcohol-dependent White Italians; 33% smokers; 0% polysubstance abusers; non-alcohol-dependent by same questionnaire as for cases; $n = 64$ (51 men, 13 women); mean age, 45 (SD, 9.51) years	*Taq1A* and *Taq1B*, PCR; genotyping in duplicate; no information on blinding	0.412
Samochowiec, 2000 (70)	Unrelated, Caucasian, German alcoholic patients recruited from two hospitals; ICD-10; structured clinical interview; $N = 292$ (233 men, 50 women); mean age, 43 (SD, 9) years	Ethnically matched, unrelated, nonalcoholics without neuropsychiatric disorders; no details on how controls were diagnosed or screened; $N = 192$ (70 men, 76 women); mean age, 54 (SD, 16) years	−141C ins/del, exon 8 AG, *Taq1A*, PCR; no information on blinding	1.000
Sander, 1999 (61)	Unrelated, German, alcohol-dependent individuals diagnosed according to ICD-10 and structured interview; no comorbid substance dependence bar nicotine; $N = 310$ (261 men, 49 women); mean age, 42.4 (SD, 9.4) years	Healthy volunteers; no history of previous psychiatric treatment or addictive disorder; questionnaire; $N = 196$ (108 men, 88 women); mean age, 44.3 (SD, 8.9) years	−141 C Ins/del and *Taq11A*, PCR; no information on blinding	0.893
Schwab, 1991 (47)	Alcohol dependents in detoxification; Caucasians living in Germany; diagnosis by clinical examination; $n = 45$	$n = 69$	*Taq1A*; no information on blinding	Not tested
Shaikh, 2001 (78)	Alcohol dependents from southern India admitted to a mental health hospital; SCAN* criteria and ICD-10 for alcohol dependence (Schedules for Assessment of Neuropsychiatry) plus interview; age at dependence <25 years, ≥2 first-degree relatives with alcohol dependence; $n = 50$	Hospital staff and blood donors matched on gender and ethnicity; aged >30 years; no alcohol dependence (blood donors are screened for alcohol and drug abuse, excluding tobacco); $n = 53$	*Taq1A*, PCR; no information on blinding	0.124
Suarez, 1994 (62)	Unrelated Caucasian alcoholics, mainly probands in an ongoing study of genetics and alcoholism; alcoholism determined according to Feigner criteria, structured clinical interview, and medical record review; $n = 88$	Race-matched controls all unrelated and all psychiatrically normal from three sources: spouses of alcoholics ($n = 14$), subjects in a bipolar study ($n = 12$), and subjects from a catchment's area survey; interview ($n = 63$); $n = 89$; aged 40–55 years	*Taq1A*, phD2-254 *Taq1B*, phD2-248 BclI, phD2-244 *Taq1*, phD2-249 HincII, hybridization; genotype by two independent raters blind to clinical status	0.708
Young, 2002 (44)	Unrelated male Caucasian PTSD* patients (Australian armed forces veterans) consuming harmful alcohol levels; interviewers blinded to case-control status; assessed for PTSD; interviewed for alcohol consumption; $n = 38$, mean age, 52 (SEM, 0.7) years	Group 1: unrelated male Caucasian PTSD patients (Australian armed forces veterans) consuming nonharmful alcohol levels, $n = 53$; group 2: unrelated male Australian Caucasians recruited in local hospitals and assessed for alcohol consumption, tobacco use, drug use, and family history of alcoholism, none consuming harmful alcohol levels; $n = 51$	*Taq1A*; no information on blinding	0.188

* DSM-III-R, *Diagnostic and Statistical Manual of Mental Disorders*, Third Edition, Revised; PCR, polymerase chain reaction; DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Revised; M-CIDI, Munchner Composite International Diagnostic Interview; ASI, Addiction Severity Index; SEM, standard error of the mean; NIAAA, National Institute on Alcohol Abuse and Alcoholism; RDC, research diagnostic criteria; NNRB, National Neurological Research Bank; CEPH, Centre d'etude du Polymorphisme Humain; VA, Veterans Administration; SD, standard deviation; MAST, Michigan Alcohol Screening Test; AUDIT, Alcohol Use Disorders Interview Test; ICD-10, *International Statistical Classification of Diseases and Related Health Problems*, Tenth Revision; SADS-L, Schedule for Affective Disorders and Schizophrenia, Lifetime Version; STR, short tandem repeat; AA, Alcoholics Anonymous; CNS, central nervous system; KAST, Kurihama Alcoholism Screening Test; SAAST, Self-Administered Alcohol Screening Test; SCAN, Schedule for Assessment of Neuropsychiatry; PTSD, posttraumatic stress disorder.

TABLE 2.   Effect estimates in studies that reported use of a control group matched for ethnicity

Subgroup	No. of studies	OR* dominant†	95% CI*	I^2%‡	No. of studies	OR recessive§	95% CI	I^2%‡
Caucasian	11	1.26	1.02, 1.56	37.0	11	1.26	0.84, 1.91	0
Native American	1	0.95	0.57, 1.58		1	0.83	0.56, 1.24	
Japanese	4	1.12	0.71, 1.77	69.3	4	1.25	0.89, 1.76	0
Chinese	5	1.35	1.04, 1.75	0	5	1.41	1.00, 2.00	0
Hispanic	2	1.07	0.73, 1.57	0	2	1.31	0.78, 2.20	19.5
Korean	1	1.10	0.58, 2.06		1	0.96	0.42, 2.20	
Indian	1	0.84	0.36, 1.95		1	1.25	0.42, 3.75	
Polynesian	1	0.97	0.48, 1.96		1	0.99	0.39, 2.49	
Overall	26	1.18	1.04, 1.34	22.3	26	1.18	1.01, 1.39	0

* OR, odds ratio; CI, confidence interval.
† A1A1 + A1A2 vs. A2A2 genotype.
‡ Variability between studies due to heterogeneity rather than chance.
§ A1A1 vs. A1A2 + A2A2 genotype.

alcohol dependency with being homozygote for the *A1* allele (Web figure 2). The combined odds ratio was 1.22 (95 percent CI: 1.05, 1.43) using random effects this time, with no statistical heterogeneity detected between studies ($I^2 = 0$ percent, $p = 0.92$). Again, subgroup analyses showed no notable differences between different populations, with a significant association detected in the Caucasian subgroup (OR = 1.40, 95 percent CI: 1.03, 1.91) with no heterogeneity ($I^2 = 0$ percent, $p = 0.92$) and in the Chinese subgroup (OR = 1.41, 95 percent CI: 1.00, 2.00) with no heterogeneity ($I^2 = 0$ percent, $p = 0.52$).

Sensitivity analyses

Statistically significant departures from Hardy-Weinberg equilibrium were detected in three studies (46, 68, 74). Exclusion of these three studies from both analyses did not change the overall effect for the recessive model (OR = 1.22, 95 percent CI: 1.05, 1.43; $I^2 = 0$ percent, $p = 0.89$) or remove heterogeneity between studies for the dominant model (OR = 1.38, 95 percent CI: 1.20, 1.60; $I^2 = 53.1$ percent, $p < 0.0001$). There was a slight increase in heterogeneity in the Caucasian subgroup ($I^2 = 59.7$ percent) with removal of the two studies, with significant departures from Hardy-Weinberg equilibrium.

An overall significant association was still detected using the dominant model in analyses restricted to studies reporting use of ethnic matching of controls (table 2), blinding (table 3), and screening of the control group to exclude alcohol dependents (table 4). Sensitivity analyses had little impact on heterogeneity, with the exception of the Caucasian subgroup when the analysis was restricted to studies using ethnically matched controls, where it was markedly reduced (37 percent, table 2). Similarly, for the recessive model, significant associations were still detected when restricting the analyses to studies with ethnically matched controls (table 3) and screened controls (table 4), with negligible heterogeneity.

Results for the cumulative meta-analysis after each study from 1990 to 2004, and for each genetic model, are shown in Web figures 3 and 4. For the dominant model, the cumulative meta-analysis shows that, with each additional study, although being significantly greater than one since 1990, the magnitude of the effect decreased over time; the odds ratio came closer to one and remained relatively stable beginning in 2000. For *A1* homozygotes versus both other genotypes (recessive model), the odds ratio became greater than one after 1991 and has remained so since then, reaching significance in 2001. The pooled odds ratio has changed very little since 2001.

TABLE 3.   Effect estimates in studies that reported use of blinding measures

Subgroup	No. of studies	OR* dominant†	95% CI*	I^2%‡	No. of studies	OR recessive§	95% CI	I^2%
Caucasian	11	1.76	1.25, 2.48	50.6	11	1.29	0.69, 2.40	0%
Native American	1	0.95	0.57, 1.58		1	0.83	0.56, 1.24	
Japanese	1	0.83	0.46, 1.48		1	1.95	0.85, 4.44	
Overall	13	1.54	1.12, 2.12	57.9	13	1.05	0.77, 1.43	0%

* OR, odds ratio; CI, confidence interval.
† A1A1 + A1A2 vs. A2A2 genotype.
‡ Variability between studies due to heterogeneity rather than chance.
§ A1A1 vs. A1A2 + A2A2 genotype.

TABLE 4. Effect estimates in studies that reported screening for alcohol dependency in the control group

Subgroup	No. of studies	OR* dominant†	95% CI*	I^2%‡	No. of studies	OR recessive§	95% CI	I^2%
Caucasian	24	1.54	1.24, 1.91	59.7	24	1.44	1.02, 2.02	0
Native American	1	0.95	0.57, 1.58		1	0.83	0.56, 1.24	
Japanese	4	1.12	0.71, 1.77	69.3	4	1.25	0.89, 1.76	0
Chinese	4	1.30	0.97, 1.74	0	4	1.31	0.88, 1.93	0
Hispanic	2	1.07	0.73, 1.57	0	2	1.31	0.78, 2.20	19.5
Korean	1	1.10	0.58, 2.06		1	0.96	0.42, 2.20	
Indian	1	0.84	0.36, 1.95		1	1.25	0.42, 3.75	
Polynesian	1	0.97	0.48, 1.96		1	0.99	0.39, 2.49	
Overall	38	1.33	1.15, 1.55	51.5	38	1.20	1.02, 1.41	0

* OR, odds ratio; CI, confidence interval.
† *A1A1* + *A1A2* vs. *A2A2* genotype.
‡ Variability between studies due to heterogeneity rather than chance.
§ *A1A1* vs. *A1A2* + *A2A2* genotype.

DISCUSSION

This large meta-analysis of mainly case-control studies found a small but significant association of the *Taq1A* polymorphism with alcohol dependency in both a dominant and a recessive model of gene action. Given the modest effect size found by our meta-analysis, many of the individual studies were obviously underpowered. However, there were substantial variations between studies, particularly in the Caucasian subgroup. The observed heterogeneity could be due to differences in how the samples were selected and screened or to methods of genotyping or interaction with other risk factors.

We tested whether genotype frequencies in the control groups were in agreement with Hardy-Weinberg equilibrium because it has been reported that this is miscalculated in many studies (88), and departure from Hardy-Weinberg equilibrium may point to genotyping error or other biases resulting in heterogeneity and misleading results (89). Exclusion of the three studies with significant deviation from Hardy-Weinberg equilibrium did not account for the heterogeneity or significantly change the pooled estimate. For many studies, although significant deviations were not found, agreement with Hardy-Weinberg equilibrium cannot be implied because there may have been insufficient information to detect a difference.

All of the studies had one or more serious methodological shortcomings: lack of matching for or adequate description of ethnicity of the control group, lack of blinding, inconsistent screening of control groups for alcohol dependence, inadequate description of genotyping methods, and potential variations in case definition due to imprecision of the different diagnostic criteria used. Selection of the control groups was especially problematic and in many studies included convenience samples rather than population-based controls. Population stratification cannot be ruled out because ethnicity was self-reported, and self-reported ancestry has been shown to be unreliable in outbred populations such as in the United States (90). One or more of these factors may have led to the observed heterogeneity in our meta-analysis. It is likely that each of these biases would affect the overall pooled estimate to a different extent, but it is unclear whether they would all work to bias the result in the same direction.

We investigated the impact of including studies with a greater likelihood of bias on the overall estimate by conducting a series of sensitivity analyses. Overall pooled estimates or heterogeneity values did not change markedly when potentially biased studies were excluded; however, heterogeneity was reduced substantially in the Caucasian subgroup and the overall group when the analysis was restricted to studies with ethnically matched controls.

An additional factor that has been cited as a probable cause of heterogeneity between studies is severity of alcohol dependency. We were unable to investigate the impact of severity of alcohol dependency on the pooled estimate because there was no consistent use of the term "severe." Medical complications, withdrawal symptoms, and Michigan Alcohol Screening Test scores were all used as criteria for defining the cases as severe. Severity of illness may reflect the duration of illness if based on an accumulation of complications and thus be unreliable.

The cumulative meta-analysis showed that, as more studies were published, the pooled odds ratio approached one, suggesting that one reason earlier studies showed strong associations may be chance because of small sample sizes. This finding is in agreement with an investigation of 55 meta-analyses of genetic association studies of various disease outcomes that showed that positive findings of early studies do not adequately predict establishment of an association (91).

Although we made a considerable effort to find published studies, the possibility of publication bias cannot be overlooked. It is difficult to predict the likely effect on the estimate. Most frequently, publication bias is due to preferential publication of favorable results, which tends to overestimate effects or associations; however, because of the polarity of opinion on the role of *Taq1A*, studies showing no association may not have been suppressed. We did not formally investigate publication bias through the use of funnel plots

because it is unclear whether the assumptions for their interpretation are valid when observational studies, rather than randomized controlled trials, are pooled (92, 93).

Authors of previous reviews, meta-analyses, and primary studies have presented contrasting findings on the role of *Taq1A* and alcoholism. Reviews by Noble et al. (7, 48, 94, 95) reported on meta-analyses, mainly in Caucasian populations, and concluded that there is a strong association, particularly among people with ''severe'' alcohol dependency. These analyses were all based on comparing allele frequencies in people with alcohol dependency and nonalcoholic controls, analogous to the codominant model. This type of analysis tends to produce spurious associations or overinflate *p* values because of doubling of the sample size. It is also not clear whether, in these reviews, correct methods for pooling frequencies were used, which weights each study appropriately, or whether frequencies were pooled simply by adding them all and treating the data as if they were all from one large study.

A more cautious conclusion was drawn by Gelernter et al. (10), whose meta-analysis of studies published up to 1993 failed to confirm that a strong association was found. They suggested that factors contributing to the heterogeneity were lack of careful screening to exclude problem alcohol use by the control groups and lack of matching for ethnicity rather than the use of simple racial matching.

Although the modest strength of association found in this study is of a similar magnitude to odds ratios reported for meta-analyses of other candidate genes and mental health disorders (96, 97), because of the numerous potential sources of bias in the primary studies, overestimation of the association cannot be ruled out.

While there is convincing evidence for a genetic contribution to alcohol dependence derived from family, twin, and adoption studies, it cannot be explained by a single gene operating under Mendelian laws of inheritance.

Therefore, a single gene predisposing to alcoholism is not anticipated, and the likelihood is that many, of relatively modest influence, interact with environmental factors and operate during a process of development. As such, the relatively small effect identified by this study regarding the contribution of the *A1* allele or another genetic variant linked to it is much more in keeping with the current understanding of the genetic contribution to such complex disorders and behaviors.

ACKNOWLEDGMENTS

REFERENCES

1. Turner E, Ewing J, Shilling P, et al. Lack of association between an RFLP near the D2 dopamine receptor gene and severe alcoholism. Biol Psychiatry 1992;31:285–90.
2. Bowirrat A, Oscar-Berman M. Relationship between dopaminergic neurotransmission, alcoholism, and reward deficiency syndrome. Am J Med Genet B Neuropsychiatr Genet 2005;132: 29–37.
3. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 2004;23: 540–5.
4. Blum K, Noble EP, Sheridan PJ, et al. Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA 1990;263:2055–60.
5. Lawford BR, Young RM, Rowell JA, et al. Association of the D2 dopamine receptor A1 allele with alcoholism: medical severity of alcoholism and type of controls. Biol Psychiatry 1997;41:386–93.
6. Neiswanger K, Hill SY, Kaplan BB. Association and linkage studies of the TAQI A1 allele at the dopamine D2 receptor gene in samples of female and male alcoholics. Am J Med Genet 1995;60:267–71.
7. Noble EP. Addiction and its reward process through polymorphisms of the D2 dopamine receptor gene: a review. Eur Psychiatry 2000;15:79–89.
8. Ball D. Genetic approaches to alcohol dependence. Br J Psychiatry 2004;185:449–51.
9. Buckland PR. Genetic association studies of alcoholism—problems with the candidate gene approach. Alcohol Alcohol 2001;36:99–103.
10. Gelernter J, Goldman D, Risch N. The A1 allele at the D2 dopamine receptor gene and alcoholism. A reappraisal. JAMA 1993;269:1673–7.
11. Persico AM, Bird G, Gabbay FH, et al. D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: enhanced frequencies in psychostimulant-preferring polysubstance abusers. Biol Psychiatry 1996;40:776–84.
12. Noble EP, Blum K, Khalsa ME, et al. Allelic association of the D2 dopamine receptor gene with cocaine dependence. Drug Alcohol Depend 1993;33:271–85.
13. Gelernter J, Kranzler H, Satel SL. No association between D2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans. Biol Psychiatry 1999;45:340–5.
14. Messas G, Meira-Lima I, Turchi M, et al. Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence. Psychiatr Genet 2005;15:171–4.
15. Lawford BR, Young RM, Noble EP, et al. The D(2) dopamine receptor A(1) allele and opioid dependence: association with heroin use and response to methadone treatment. Am J Med Genet 2000;96:592–8.
16. Chen CK, Hu X, Lin SK, et al. Association analysis of dopamine D2-like receptor genes and methamphetamine abuse. Psychiatr Genet 2004;14:223–6.
17. Tsai SJ, Cheng CY, Shu LR, et al. No association for D2 and D4 dopamine receptor polymorphisms and methamphetamine abuse in Chinese males. Psychiatr Genet 2002;12:29–33.
18. Munafo M, Clark T, Johnstone E, et al. The genetic basis for smoking behavior: a systematic review and meta-analysis. Nicotine Tob Res 2004;6:583–97.
19. Comings DE, Rosenthal RJ, Lesieur HR, et al. A study of the dopamine D2 receptor gene in pathological gambling. Pharmacogenetics 1996;6:223–34.
20. Gelernter J, Southwick S, Goodson S, et al. No association between D2 dopamine receptor (DRD2) ''A'' system alleles, or DRD2 haplotypes, and posttraumatic stress disorder. Biol Psychiatry 1999;45:620–5.
21. Comings DE, Muhleman D, Gysin R. Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. Biol Psychiatry 1996;40:368–72.

22. Ball D, Collier D. Substance misuse. Oxford, United Kingdom: Oxford University Press, 2002.
23. Loh EW, Higuchi S, Matsushita S, et al. Association analysis of the GABA(A) receptor subunit genes cluster on 5q33-34 and alcohol dependence in a Japanese population. Mol Psychiatry 2000;5:301–7.
24. Loh EW, Smith II, Murray R, et al. Association between variants at the GABAAbeta2, GABAAalpha6 and GABAA-gamma2 gene cluster and alcohol dependence in a Scottish population. Mol Psychiatry 2000;5:452.
25. Sander T, Ball D, Murray R, et al. Association analysis of sequence variants of GABA(A) alpha6, beta2, and gamma2 gene cluster and alcohol dependence. Alcohol Clin Exp Res 1999;23:427–31.
26. Chang YT, Sun HS, Fann CS, et al. Association of the gamma-aminobutyric acid A receptor gene cluster with alcohol dependence in Taiwanese Han. Mol Psychiatry 2002;7:828–9.
27. Parsian A, Zhang ZH. Human chromosomes 11p15 and 4p12 and alcohol dependence: possible association with the GABRB1 gene. Am J Med Genet 1999;88:533–8.
28. Song J, Koller DL, Foroud T, et al. Association of GABA(A) receptors and alcohol dependence and the effects of genetic imprinting. Am J Med Genet B Neuropsychiatr Genet 2003;117:39–45.
29. Noble EP, Zhang X, Ritchie T, et al. D2 dopamine receptor and GABA(A) receptor beta3 subunit genes and alcoholism. Psychiatry Res 1998;81:133–47.
30. Caetano R, Cunradi C. Alcohol dependence: a public health perspective. Addiction 2002;97:633–45.
31. Grant BF, Stinson FS, Harford TC. Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: a 12-year follow-up. J Subst Abuse 2001;13:493–504.
32. Barnes GM, Welte JW. Patterns and predictors of alcohol use among 7-12th grade students in New York State. J Stud Alcohol 1986;47:53–62.
33. Warner LA, White HR. Longitudinal effects of age at onset and first drinking situations on problem drinking. Subst Use Misuse 2003;38:1983–2016.
34. Humphrey JA, Friedman J. The onset of drinking and intoxication among university students. J Stud Alcohol 1986;47:455–8.
35. Hasin DS, Glick H. Severity of DSM-III-R alcohol dependence: United States, 1988. Br J Addict 1992;87:1725–30.
36. Grant BF, Dawson DA. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. J Subst Abuse 1997;9:103–10.
37. King KM, Chassin L. A prospective study of the effects of age of initiation of alcohol and drug use on young adult substance dependence. J Stud Alcohol Drugs 2007;68:256–65.
38. Pitkanen T, Lyyra AL, Pulkkinen L. Age of onset of drinking and the use of alcohol in adulthood: a follow-up study from age 8-42 for females and males. Addiction 2005;100:652–61.
39. Cloninger CR, Bohman M, Sigvardsson S. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch Gen Psychiatry 1981;38:861–8.
40. Zucker RA, Ellis DA, Fitzgerald HE. Developmental evidence for at least two alcoholisms. I. Biopsychosocial variation among pathways into symptomatic difficulty. Ann N Y Acad Sci 1994;708:134–46.
41. Caetano R, Tam T, Greenfield T, et al. DSM-IV alcohol dependence and drinking in the U.S. population: a risk analysis. Ann Epidemiol 1997;7:542–9.
42. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
43. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
44. Young RM, Lawford BR, Noble EP, et al. Harmful drinking in military veterans with post-traumatic stress disorder: association with the D2 dopamine receptor A1 allele. Alcohol Alcohol 2002;37:451–6.
45. Goldman D, Urbanek M, Guenther D, et al. Linkage and association of a functional DRD2 variant [Ser311Cys] and DRD2 markers to alcoholism, substance abuse and schizophrenia in Southwestern American Indians. Am J Med Genet 1997;74:386–94.
46. Bolos AM, Dean M, Lucas-Derse S, et al. Population and pedigree studies reveal a lack of association between the dopamine D2 receptor gene and alcoholism. JAMA 1990;264:3156–60.
47. Schwab S, Soyka M, Neiderecker M. Allelic association of human dopamine D2-receptor DNA polymorphism ruled out in 45 alcoholics. Am J Hum Genet 1991;49:203.
48. Noble EP. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am J Med Genet 2003;116b:103–25.
49. Foley PF, Loh EW, Innes DJ, et al. Association studies of neurotransmitter gene polymorphisms in alcoholic Caucasians. Ann N Y Acad Sci 2004;1025:39–46.
50. Anghelescu I, Germeyer S, Muller MJ, et al. No association between the dopamine d2 receptor taqi a1 allele and earlier age of onset of alcohol dependence according to different specified criteria. Alcohol Clin Exp Res 2001;25:805–9.
51. Arinami T, Itokawa M, Komiyama T, et al. Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. Biol Psychiatry 1993;33:108–14.
52. Cook BL, Wang ZW, Crowe RR, et al. Alcoholism and the D2 receptor gene. Alcohol Clin Exp Res 1992;16:806–9.
53. Cruz C, Camarena B, Mejia JM, et al. The dopamine D2 receptor gene TaqI A1 polymorphism and alcoholism in a Mexican population. Arch Med Res 1995;26:421–6.
54. Finckh U, von Widdern O, Giraldo-Velasquez M, et al. No association of the structural dopamine D2 receptor (DRD2) variant 311Cys with alcoholism. Alcohol Clin Exp Res 1996;20:528–32.
55. Geijer T, Neiman J, Rydberg U, et al. Dopamine D2-receptor gene polymorphisms in Scandinavian chronic alcoholics. Eur Arch Psychiatry Clin Neurosci 1994;244:26–32.
56. Gelernter J, O'Malley S, Risch N, et al. No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. JAMA 1991;266:1801–7.
57. Gorwood P, Batel P, Gouya L, et al. Reappraisal of the association between the DRD2 gene, alcoholism and addiction. Eur Psychiatry 2000;15:90–6.
58. Heinz A, Sander T, Harms H, et al. Lack of allelic association of dopamine D1 and D2 (TaqIA) receptor gene polymorphisms with reduced dopaminergic sensitivity to alcoholism. Alcohol Clin Exp Res 1996;20:1109–13.
59. Ishiguro H, Arinami T, Saito T, et al. Association study between the -141C Ins/Del and TaqI A polymorphisms of the dopamine D2 receptor gene and alcoholism. Alcohol Clin Exp Res 1998;22:845–8.
60. Lu RB, Lee JF, Ko HC, et al. Dopamine D2 receptor gene (DRD2) is associated with alcoholism with conduct disorder. Alcohol Clin Exp Res 2001;25:177–84.
61. Sander T, Ladehoff M, Samochowiec J, et al. Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. Alcohol Clin Exp Res 1999;23:578–81.

62. Suarez BK, Parsian A, Hampe CL, et al. Linkage disequilibria at the D2 dopamine receptor locus (DRD2) in alcoholics and controls. Genomics 1994;19:12–20.

63. Bau CH, Almeida S, Hutz MH. The TaqI A1 allele of the dopamine D2 receptor gene and alcoholism in Brazil: association and interaction with stress and harm avoidance on severity prediction. Am J Med Genet 2000;96:302–6.

64. Chen WJ, Lu ML, Hsu YP, et al. Dopamine D2 receptor gene and alcoholism among four aboriginal groups and Han in Taiwan. Am J Med Genet 1997;74:129–36.

65. Comings DE, Comings BG, Muhleman D, et al. The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA 1991;266:1793–800.

66. Goldman D, Dean M, Brown GL, et al. D2 dopamine receptor genotype and cerebrospinal fluid homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenyl-glycol in alcoholics in Finland and the United States. Acta Psychiatr Scand 1992;86:351–7.

67. Lee MS, Lee KJ, Kwak DI. No association between the dopamine D2 receptor gene and Korean alcoholism. Psychiatr Genet 1997;7:93–5.

68. Lu RB, Ko HC, Chang FM, et al. No association between alcoholism and multiple polymorphisms at the dopamine D2 receptor gene (DRD2) in three distinct Taiwanese populations. Biol Psychiatry 1996;39:419–29.

69. Ovchinnikov IV, Druzina E, Ovtchinnikova O, et al. Polymorphism of dopamine D2 and D4 receptor genes and Slavic-surnamed alcoholic patients. Addict Biol 1999;4:399–404.

70. Samochowiec J, Ladehoff M, Pelz J, et al. Predominant influence of the 3′-region of dopamine D2 receptor gene (DRD2) on the clinical phenotype in German alcoholics. Pharmacogenetics 2000;10:471–5.

71. Lee JF, Lu RB, Ko HC, et al. No association between DRD2 locus and alcoholism after controlling the ADH and ALDH genotypes in Chinese Han population. Alcohol Clin Exp Res 1999;23:592–9.

72. Blum K, Noble EP, Sheridan PJ, et al. Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. Alcohol 1991;8:409–16.

73. Kono Y, Yoneda H, Sakai T, et al. Association between early-onset alcoholism and the dopamine D2 receptor gene. Am J Med Genet 1997;74:179–82.

74. Karaoguz MY, Onen I, Cosar B, et al. Lack of association between TaqIA RFLP of the dopamine D2 receptor gene and alcoholism in type II Turkish alcoholics. Korean J Genet 2004;26:149–54.

75. Parsian A, Todd RD, Devor EJ, et al. Alcoholism and alleles of the human D2 dopamine receptor locus. Studies of association and linkage. Arch Gen Psychiatry 1991;48:655–63.

76. Pastorelli R, Bardazzi G, Saieva C, et al. Genetic determinants of alcohol addiction and metabolism: a survey in Italy. Alcohol Clin Exp Res 2001;25:221–7.

77. Matsushita S, Muramatsu T, Murayama M, et al. Alcoholism, ALDH2*2 allele and the A1 allele of the dopamine D2 receptor gene: an association study. Psychiatry Res 2001;104:19–26.

78. Shaikh KJ, Naveen D, Sherrin T, et al. Polymorphisms at the DRD2 locus in early-onset alcohol dependence in the Indian population. Addict Biol 2001;6:331–5.

79. Limosin F, Gorwood P, Loze JY, et al. Male limited association of the dopamine receptor D2 gene TaqI a polymorphism and alcohol dependence. Am J Med Genet 2002;112:343–6.

80. Amadeo S, Abbar M, Fourcade ML, et al. D2 dopamine receptor gene and alcoholism. J Psychiatr Res 1993;27:173–9.

81. Chen CH, Chien SH, Hwu HG. Lack of association between TaqI A1 allele of dopamine D2 receptor gene and alcohol-use disorders in atayal natives of Taiwan. Am J Med Genet 1996;67:488–90.

82. Cruz C, Diaz A, Taba Shiwa S. Analysis of Taql A1 polymorphism of dopamine D2 receptor gene in alcoholics and no alcoholics with and without severe hepatic damage. Salud Ment 1997;20:6–11.

83. Gelernter J, Kranzler H. D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. Neuropsychopharmacology 1999;20:640–9.

84. Hietala J, Pohjalainen T, Heikkila-Kallio U, et al. Allelic association between D2 but not D1 dopamine receptor gene and alcoholism in Finland. Psychiatr Genet 1997;7:19–25.

85. Noble EP, Syndulko K, Fitch RJ, et al. D2 dopamine receptor TaqI A alleles in medically ill alcoholic and nonalcoholic patients. Alcohol Alcohol 1994;29:729–44.

86. Amadeo S, Noble EP, Fourcade Amadeo ML, et al. Association of D2 dopamine receptor and alcohol dehydrogenase 2 genes with Polynesian alcoholics. Eur Psychiatry 2000;15:97–102.

87. Konishi T, Luo HR, Calvillo M, et al. ADH1B*1, ADH1C*2, DRD2 (-141C Ins), and 5-HTTLPR are associated with alcoholism in Mexican American men living in Los Angeles. Alcohol Clin Exp Res 2004;28:1145–52.

88. Salanti G, Amountza G, Ntzani EE, et al. Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. Eur J Hum Genet 2005;13:840–8.

89. Trikalinos TA, Salanti G, Khoury MJ, et al. Impact of violations and deviations in Hardy-Weinberg equilibrium on postulated gene-disease associations. Am J Epidemiol 2006;163:300–9.

90. Burnett MS, Strain KJ, Lesnick TG, et al. Reliability of self-reported ancestry among siblings: implications for genetic association studies. Am J Epidemiol 2006;163:486–92.

91. Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG, et al. Establishment of genetic associations for complex diseases is independent of early study findings. Eur J Hum Genet 2004;12:762–9.

92. Egger M, Davey Smith G, Schneider M. Systematic reviews of observational studies. In: Egger M, Davey Smith G, Altman DG, eds. Systematic reviews in health care. London, United Kingdom: BMJ Publishing Group, 2001.

93. Little J, Higgins JPT, eds. The HuGENet™ HuGE review handbook, version 1.0. (http://www.hugenet.ca) (accessed February 28, 2006).

94. Noble EP. The D2 dopamine receptor gene: a review of association studies in alcoholism. Behav Genet 1993;23:119–29.

95. Pato CN, Macciardi F, Pato MT, et al. Review of the putative association of dopamine D2 receptor and alcoholism: a meta-analysis. Am J Med Genet 1993;48:78–82.

96. Maher BS, Marazita ML, Ferrell RE, et al. Dopamine system genes and attention deficit hyperactivity disorder: a meta-analysis. Psychiatr Genet 2002;12:207–15.

97. Lohmueller KE, Pearce CL, Pike M, et al. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177–82.

98. Gelernter J, Kranzler H, Cubells JF, et al. DRD2 allele frequencies and linkage disequilibria, including the –141CIns/Del promoter polymorphism, in European-American, African-American, and Japanese subjects. Genomics 1998;51:21–6.